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Screening of mycotoxigenic *Aspergillus* in poultry feed of Telangana, India

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Abstract
The main source of fungal microflora is originate from materials of plant origin, primarily cereals and feed samples. In relation to humans and animals, mycotoxins exhibit toxic action and are characterized by carcinogenic, mutagenic, teratogenic and estrogenic properties. Due to the diversity of toxic effects, as well as their resistance to the action of high tempareture, the presence of mycotoxins in feeds constitutes a potential threat to human and animal health. Incidence of diffent species of *Aspergillus* were analyzed and isolated from 87 poultry feed samples (starter, breeder, boiler and layer) collected from different places of Telangana region. In all samples, 357 strains representing elevan species of *Aspergillus* such as *A. flavus, A. flavipus, A. fumigatus, A. glucus, A. niger, A. nidulans, A. ochreceus, A. parasiticus, A. terreus, A. ustus* and *A. versicolor* were probably recorded. All the feed samples were contaminated with a number of different fungal species. These 357 strains of *Aspergillus* were screened for mycotoxin production by Thin Layer Chromatography (TLC) using different spray reagents. Among them, 165 strains representing 11 species were recorded to be positive for production of aflatoxin, oxalic acid, ochratoxin A, patulin, aflatrem, gliotoxin, nidulotoxin, citrinin and sterigmatocystin etc.

Keywords: *Aspergillus* species, poultry feed, mycotoxins

Introduction
Food is both the cornerstone of life and its protection, and so far most people pay little attention to certifying that food is safe to consume. It is typically the potential problems with pesticides or other man-made chemicals when food safety concerns are posed. Mycotoxins formed by fungal species, however, are strong mutagens and carcinogens and are therefore an equal or greater threat to food safety (Krska *et al.*, 2008; Shepherd, 2008) [2, 8]. In food production world wide, food safety is necessary. In the healthy food chain, po ultry meat, eggs and polutry products derived from them are important. As far as safety is concerned, particular attention is paid to the potential contamination of food and poultry fe ed with fungi and the possibility of mycotoxin contaminatio n (Haque.M.A. et al., 2020) [5]. In the production of poultry, feed is the important factor. There is a growing concern of the role of mycotoxins (aflatrem, aflatoxin, sterigmatocystin, gliotoxin, oxalic acid, citrinin, patulin, nidulotoxin and ochratoxin) on humans and livestock health. Being temperate regions in India, the environmental conditions especially temperature and relative humidity may be responsible for contamination of food grains with high level of mycotoxins. Therefore, there is a need to bring out the awareness on mycotoxins among the farmers in India.The present study was done to study the incidence of *Aspergillus* species from different poultry feeds.

Materials and Methods
The screening and incidence of different poultry feeds was similar to the method adopted by Rao et al (2015) [6]. The different *Aspergillus* species isolated were analysed for mycotoxins production by employing thin layer chromatography (TLC) method (Lund, 1995) [3]. Well cleaned and dried glass plates were used for coating the silicagel. Silica gel and water 70:30 (w/v) ratio was taken in a stopped round bottle and shaken thoroughly for 5 min to make uniform slurry.
Then applied this mixture on glass plates with uniform thickness 2mm with TLC applicator. The TLC plates thus coated were allowed to dry for 3 to 4 hrs and then activated by heating at 80-90˚C for 2 hrs just before use. The plates were then submerged in 10% solution of oxalic acid in methanol solution for 10 min at 110˚C. The mycotoxin extract of about 10 µL was spotted immediately on to the activated and cooled TLC palte by using micro-liters syringe or disposable capillary tubing pipettes, care was taken to keep the spot size to minimum and uniform along and imaginary line approximately 1.5-2.8 cm from the bottom edge of activated and cooled TLC plates. After application the plates were developed in a suitable solvent system (toluene: ethyl acetate: formic acid in 6:3:1 v/v/v ratio) (Samson and pitt, 2000) [7]. Mycotoxin production by *Aspergillus* species were identified either by the colour of the flouresence using spray reagents under U.V spectrum (U.V-10VIS) at 333nm or by the Rf value (Pitt and Hacking, 1997; Frivad *et al.*, 2004). The Rf value was calculated by the following formula:

Rf = Distance travelled by the compound /Distance travelled by the solvent.

Results and Discussion
The incidence of fungal species from poultry feed samples collected from Khammam, Warangal, Karimnagar and Adilabad District of Telangana State were analyzed and the results are precised in Table 1.
The above table is evident that the mycoflora varied both quantitatively and qualitatively with the product, place and time of collection. Three fifty seven strains belongs to elevan fungal species were recorded from different poultry

feed samples of Telangana region. Among them *A. flavus* was recorded with highest percentage of incidence, frequency and abundance in all samples collected from Adilabad followed Warangal, Karimnagar and Khammam Districts of Telangana. *A. ochreceous* and *A. parasiticus* were recorded with same percentage of frequency and abundance but differ in their incidences in all the samples collected from 4 districts. A. versicolor were recorded with least percentage of incidence, frequency and abundance in all the poultry samples collected from Khammam, Warangal, Karimnagar and Adilabad Districts of Telangana region. In poultry feed samples collected from Khammam District were recorded with highest percentage of incidence of *A. flavus* followed by *A.*

terreus in decreasing order but differ in their percentage of frequency and abundance. Whereas in samples collected from Warangal, Karimnagar and Adilabad District were recorded with highest percentage of incidence of *A. flavus* followed by *A. niger* but differ in their percentage of frequency and abundance. The rest of the fungal species were recorded with the intermediate percentage of incidence. Table 2 revealed that 357 strains of eleven *Asperillus* species isolated from poultry feed were screened for the detection of mycotoxins production by TLC plate method by using different spray reagents (Table 3).

Among them 165 strains were showed positive for mycotoxins production. Out of 74 strains of *Aspergillus flavus* screened, 48 strains of *A. flavus* were recorded to be positive for the production of aflatoxin and aflatrem. Thirteen strains of *A.*

fumigatus out of 31strains produce gliotoxin, whereas 8 strains out of 33 strains of *A. flavipus* were found to be positive for sterigmatocystin production. Twenty nine strains of *A. glucus* were screened for mycotoxins production, which are found to be negative for any mycotoxins production. Twelve strains of *A. niger* were positive for oxalic acid out of 47 strains. Among 27 strains of *A.*

nidulans were screened, 10 strains are found to be positive for nidulotoxin production. Sixteen strains of *A. terreus* produce patulin, out of 31 strains. Similarly, 15 strains of *A. ochreceus* produce ochratoxin out of 19 strains screened, whereas 10 strains of *A.*

parasiticus found to be positive for aflatoxin production, out of 19 strains screened for mycotoxins production. Six strains of *A. versicolor* produced sterigmatocystin out of 16 strains screened for mycotoxins production. Among 31 strains of *A. versicolor*, 27 strains are recorded to produce citrinin. The order of percentage of contamination of different poultry feeds by mycotoxins produced by *Aspergillus* species were ochratoxin, citrinin, aflatrem, aflatoxin, patulin, gliotoxin, nidulotoxin, sterigmatocystin and oxalic acid respectively. Based on above results it is clear that there is a need for routine monitoring of food grains of commerce both during storage and marketing, especially in developing countries. . The occurrence of these mycotoxins in crops of Telangana State food grains of South India interlinked with toxigenic moulds. Diversity evidences of the toxigenic species showed that the contamination was higher in the studied region.

Table 1: Incidence of Poultry Feed Samples Collected from Khammam, Warangal, Karimnagar and Adilabad Districts of Telangana State.

Organism	**Khammam**																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
A. flavus	21.7	19.2	7.14	21.0	--	20.0	19.0	--	11.1	4.70	37.5	9.09	20.0	3.2	--	71.4	--	--	22.2	13.0	2.9
A. fumigatus	--	--	42.9	--	33.3	--	--	7.10	--	--	12.5	--	--	9.67	--	2.0	2.0	--	5.5	--	5.8
A. flavipus	13.0	30.4	--	5.26	--	13.3	--	--	5.50	19.0	--	--	10.0	--	--	7.1	--	11.5	--	--	5.8
A. glucus	--	11.5	--	36.8	--	--	--	21.4	--	--	--	4.5	--	--	11.1	--	6.6	--	--	8.6	--
A. nidulance	26.0	11.5	--	--	16.7	--	19.0	--	--	9.50	--	13.6	--	12.9	--	3.06	--	3.8	22.2	--	2.9
A. niger	17.4	--	7.14	--	--	6.60	--	--	2.70	--	--	--	3.3	--	--	--	6.6	--	--	--	--
A. ochreceus	--	--	--	--	22.2	--	23.8	--	8.30	--	--	--	6.6	--	--	1.0	--	34.6	--	--	5.8
A. parasiticus	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	13.0	20.5
A. terreus	13.0	--	28.6	10.5	--	--	14.3	--	2.70	--	16.6	--	10.0	--	44.4	--	20.0	--	33.3	--	--
A. ustus	--	7.69	14.2	--	--	20.0	--	--	--	4.70	--	9.09	--	--	--	2.0	--	3.8	--	8.6	2.9
A. versicolor	--	--	--	--	--	--	--	--	--	--	--	--	--	16.1	--	--	10.0	--	--	--	--

Table 1: of Continuation

Organism	**Warangal**																								
	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
A. flavus	32.4	33.7	72.2	2.3	12.5	18.8	25	12.5	13.6	11.1	10.0	28.6	28.0	11.5	25.9	18.2	45.0	21.2	20.8	36.8	50.0	18.2	40.6	7.6	18.6
A. fumigatus	--	--	--	--	29.2	--	18.8	--	--	5.5	--	10.7	--	--	7.4	--	--	--	--	5.2	--	--	9.3	--	--
A. flavipus	--	--	5.5	--	--	12.5	--	3.57	9.09	5.5	3.3	10.7	4.0	--	3.7	6.8	--	3.0	--	--	--	--	--	--	11.6
A. glucus	5.4	--	--	--	4.1	--	--	--	--	11.1	--	--	--	3.8	--	--	--	--	12.5	--	6.25	--	--	6.9	3
A. nidulance	--	4.6	--	6.9	--	--	12.5	3.57	--	27.7	--	--	4.0	--	7.4	--	--	--	--	--	--	--	--	--	--
A. niger	--	8.1	5.5	4.6	4.1	6.25	3.1	3.57	9.09	11.1	3.3	25	4.0	3.8	7.4	11.3	35.0	3.0	4.1	15.78	--	4.5	21.9	6.41	25.6
A. ochreceus	--	--	--	4.6	--	--	--	--	4.5	--	--	--	--	11.5	--	--	10.0	--	--	--	--	--	--	--	--
A. parasiticus	--	--	--	--	--	--	--	10.7	--	--	10	--	--	--	--	--	--	12.1	--	5.2	--	--	9.3	--	6.9
A. terreus	--	2.3	--	9.3	--	--	9.3	--	--	--	6.6	--	--	--	--	4.5	--	--	10.4	--	--	9	--	--	--
A. ustus	--	5.8	--	--	8.3	9.3	--	7.1	--	--	--	--	--	--	--	--	--	9.09	--	--	--	--	--	--	--
A. versicolor	8.1	3.4	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	4.1	--	--	--	--	--	4.6

Table 1: Continuation

Organism	Karimnagar																		
	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65
A. flavus	9.3	15.3	24.4	66.6	83.3	31.5	56	--	--	--	--	--	--	23.5	67.4	84.5	3.5	--	25.0
A. fumigatus	4.6	--	--	--	6.6	--	--	--	3.0	12.0	--	--	--	--	--	--	3.5	--	8.30
A. flavipus	--	12.8	13.3	--	--	--	--	--	--	9.25	--	2.0	--	11.7	--	--	3.5	--	--
A. glucus	1.5	--	8.8	33.3	--	13.2	34.1	--	--	--	22.2	6.1	--	--	--	0.0	39.2	--	--
A. nidulance	--	--	--	--	--	--	4.8	--	--	3.7	--	--	--	23.5	--	--	--	15.7	--
A. niger	67.1	69.2	6.6	--	--	7.89	--	--	20.0	--	--	--	--	--	6.9	--	3.5	5.20	--
A. ochreceus	--	--	--	--	6.6	--	--	3	--	33.3	--	57.1	--	11.2	--	--	--	--	--
A. parasiticus	--	--	4.4	--	--	--	--	--	--	5.5	--	--	--	--	--	--	--	31.5	--
A. terreus	--	--	--	--	3.3	--	--	--	12.0	--	--	--	16.6	--	--	2.3	--	--	--
A. ustus	4.6	--	17.7	--	--	13.2	--	--	--	12.9	--	--	--	--	--	--	--	--	4.1
A. versicolor	--	--	--	--	--	10.5	--	--	20.0	--	--	6.1	--	--	4.6	--	--	--	--

Table 1: Continuation

Organism	Adilabad																					Frequency (%)	Abandence (%)
	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	
A. flavus	41.9	--	30.7	72.5	70.2	3.9	38.4	43.3	10.0	20.0	42.9	16.6	25.0	20.3	46.2	20.0	28.9	4.16	37.5	25.0	7.8	19.3	85.05	2.89
A. fumigatus	--	--	--	--	--	--	--	--	--	--	--	--	39.2	5.08	7.4	2.5	2.6	4.16	6.25	--	--	10.5	33.33	1.13
A. flavipus	--	6.25	15.3	--	--	5.8	--	--	--	--	57.1	--	--	--	--	--	--	--	--	--	5.8	--	37.93	1.29
A. glucus	--	--	--	--	6.3	--	--	13.3	--	--	--	--	--	--	1.8	--	23.7	8.33	--	15.0	--	--	32.18	1.09
A. nidulance	3.2	--	15.3	--	--	--	--	--	--	--	--	5.5	--	--	--	5.0	--	--	--	--	--	1.75	31.03	1.05
A. niger	16.1	--	--	2.19	--	11.7	--	--	20.0	31.1	--	--	14.2	--	2.7	--	--	16.6	--	--	1.9	10.5	54.02	1.83
A. ochreceus	--	18.75	--	--	--	--	23.0	--	--	--	--	--	3.5	--	--	--	--	--	--	--	--	--	21.83	0.74
A. parasiticus	--	--	15.3	--	4.2	--	--	10.0	40.0	--	--	13.8	--	--	0.9	--	--	--	--	--	1.9	3.5	21.83	0.74
A. terreus	--	--	--	3.2	--	--	--	--	--	--	--	--	3.5	61.01	32.4	37.5	26.3	66.6	31.25	55.0	43.1	49.1	36.78	1.25
A. ustus	--	18.8	--	--	--	5.8	--	--	--	15.5	--	--	--	--	2.7	--	--	--	--	--	11.7	--	27.58	0.91
A. versicolor	6.4	--	--	--	2.1	--	--	10	--	--	--	16.6	--	--	--	--	5.2	--	0.3	--	--	--	18.39	0.62

Table 2: Mycotoxins Producing Potentials of Different Strains of *Aspergillus* Species Associated with Poultry Feed

Name of the organism	No.of strains screened	Toxin producing strains	Ts (%)	Mycotoxin
Aspergillus flavus	74	48	64%	Aflatrem, Aflatoxin
A. fumigatus	31	13	41%	Gliotoxin
A. flavipus	33	8	24%	Sterigmatocystin
A. glaucus	29	-	-	-
A. niger	47	12	25%	Oxalic acid
A. nidulans	27	10	37%	Nidulotoxin
A. terreus	31	16	51%	Patulin
A. ochreceus	19	15	98%	Ochratoxin
A. parasiticus	19	10	52%	Aflatoxin
A. versicolor	16	6	37%	Sterigmatocystin
A. terreus	31	27	87%	Citrinin
Ts (%), Percentage of toxigenic strains				

Table 3: Detection *Aspergillus* Producing Mycotoxin by Different Spray Reagents

Name of the mycotoxin	Rf	Solvent system	Spray reagent	U.V	Visible
Aflatrem, Aflatoxin	0.52	T: Ea: F (6:3:1)			
Gliotoxin	0.52	T: Ea: F (6:3:1)	5	Brown	-
Oxalic acid, citrinin	0.34	T: Ea: F (6:3:1)	1,2,3	-	Yellow, Brown, Yellow, Light brown
Patulin,citrinin	0.22	T: Ea: F (6:3:1)	1,2,3	Yellow,	Yellow, Red
Ochratoxin	0.41	T: Ea: F (6:3:1)	2,3,	Bright blue,	Yellow, Purple brown
Cyclopiazonic acid	0.24	T: Ea: F (6:3:1)	1,2,3,4	Black, Red brown	-
1, $CeSo_4$ 1% in 6N H_2SO_4; 2, 2,4, DNP; 3, 3% $FeCl_3$ in ethanol; 4, Chromotropic acid, 5, 5% silver nitrate in 90 % ethanol; T, toluene; Ea, Ethyl acetate; F, Formic acid.					

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